AMENDMENTS

IN THE CLAIMS

1. (Twice Amended) A [plurality] multiplicity of single-stranded oligonucleotide DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a single multiplex polymerase chain reaction (PCR), said primers having a 5' X domain [domain, X, and a 3' domain, Y] and a 3' Y domain, wherein;

a) [said 5'-X domains] each said 5'X domain comprises a common sequence that does not hybridize to and has no homology with any one of said multiple target DNA sequences or its complement, whereby primer-primer dimer formation and the synthesis of spurious amplification products are prevented;

b) the melting temperature of a hybrid between X and its complement in the absence of other sequences is greater than about 60°C;

c) [said 3'-Y domains] each said 3'Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement whereby primer-primer dimer formation and the synthesis of spurious amplification products are prevented; and

d) the melting temperature of a hybrid between at least one of said 3'-Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement present in said multiplex PCR; and

each of said primers being capable of annealing specifically with its cognate target sequence under uniform high stringency annealing conditions during said amplification.

2. (Twice Amended) The multiplicity of single-stranded oligonucleotide DNA primers according to claim 1, wherein X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64).

3. (Twice Amended) The multiplicity of single-stranded oligonucleotide DNA primers according to claim 1, wherein X and Y each comprise from 17 to 20 bases.

4. (Twice Amended) The multiplicity of single-stranded oligonucleotide DNA

primers according to claim 1, wherein the melting temperature of a hybrid formed between each of said primers and its complement in a solution of 0.5M NaCl is at least 72°C.

5. (Twice Amended) A multiplicity of single-stranded oligonucleotide DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a single multiplex polymerase chain reaction (PCR), wherein said primers consist of the sequence 5'-GCGGTCCCAAAAGGGTCGT (SEQ ID NO:64) (Y)-3', wherein an individual Y comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement.

6. (Twice Amended) A method for simultaneous amplification of multiple [DNA] target DNA sequences present in a DNA sample, said method comprising:

a) contacting said DNA sample, in a single reaction mixture, with a multiplicity of [paired] single-stranded oligonucleotide [primers] DNA primer pairs having [structure 5'-XY-3'] a 5'X domain, and a 3'Y domain, wherein

(i) each said X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), whereby primer-primer dimer formation and the synthesis of spurious amplification products are prevented, and

(ii) each said Y domain comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement, whereby primer-primer dimer formation and the synthesis of spurious amplification products are prevented; and

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters.

7. (Twice Amended) A method for simultaneously detecting the presence of multiple [defined] target DNA sequences in a DNA sample, which comprises the steps of:

a) simultaneously contacting said DNA sample, in a single reaction mixture, with a multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said [pairs] multiplicity of single-stranded oligonucleotide DNA primer pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer, wherein

(i) said first oligonucleotide DNA primer [of each pair] has [the structure 5'-XY-3'] a 5'X domain and a 3'Y domain, wherein each said X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said second oligonucleotide DNA primer [of each pair] has [a structure 5'-XY-3'] a 5'X domain and a 3'Y domain, wherein each said X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement,

whereby primer-primer dimer formation and synthesis of spurious amplification products are prevented; and

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters to form amplification products for each of said multiple [defined] target DNA sequences [primed] amplified with said multiplicity of single-stranded oligonucleotide DNA primers; and

c) detecting [the] said amplification products.

8. (Amended) The method [of] according to claim 7, wherein detection of said amplification [product] products indicates the presence of [the] said multiple target DNA [sequence] sequences in [a] said DNA sample.

9. (Amended) The method [of] according to claim 7, wherein said step of detecting [step] comprises gel electrophoresis.

10. (Twice Amended) A method for high-throughput genetic screening to simultaneously detect the presence of multiple [defined] target DNA sequences in DNA [samples] sample(s) obtained from one or more individuals, said method[s] comprising the steps of:

a) [providing a sample of DNA from said individual(s);

b)] simultaneously contacting said DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer, wherein

(i) said first oligonucleotide DNA primer of each pair has [the structure 5'-XY-3'] a 5'X domain and a 3'Y domain, wherein each X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said second primer of each pair has [the structure 5'-XY-3',] a 5'X domain and a 3'Y domain, wherein each X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y domain comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement[;],

whereby primer-primer dimer formation, and the synthesis of spurious amplification products are prevented.

[c)] b) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same reaction conditions and cycling parameters to form amplification products for each of said multiple [defined] target DNA sequences [primed with said oligonucleotides]; and

[d)] c) detecting [the] said amplification products.

11. (Amended) The method [of] according to claim10, wherein detection of [an] said amplification [product] products indicates the presence of [the] said multiple target DNA sequence(s) in [the] said DNA sample(s).

12. (Twice Amended) The method [of] according to claim 10, wherein said step of detecting [step] comprises gel electrophoresis.

13. (Twice Amended) A method for simultaneously [amplifying and] detecting [multiple defined target sequences] amplification products of multiple target DNA sequence(s) in a DNA sample(s), said method comprising the steps of:

a) simultaneously contacting said DNA sample(s) with a [plurality] multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer each having [the structure 5'-XY-3'] a 5'X domain and a 3'Y domain, wherein

(i) said X domain in said first oligonucleotide DNA primer [of each pair] comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said X domain in said second oligonucleotide DNA primer [of each pair has the structure 5'-XY-3', wherein each X] comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and [each] said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement;

c) subjecting said sample(s) to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same conditions and cycling parameters to form amplification products for each of said multiple [defined] target DNA sequences primed with said oligonucleotides, and

d) detecting [the] said amplification products.

14. (Amended) A method of screening to simultaneously detect amplification products of multiple target DNA sequences [of interest] in DNA sample(s), [the] said method comprising the steps of:

a) [obtaining a sample of DNA to be screened for said multiple target sequences of interest,

b)] contacting said DNA sample(s) with a [plurality] multiplicity of single-stranded oligonucleotide DNA primer pairs having [the structure 5'-XY-3'] a 5'X domain, and a 3'Ydomain, under single multiplex polymerase chain reaction conditions wherein coamplification of said multiple target DNA sequences occurs in one or more cycles of identical melting,

annealing and extending temperatures and times, wherein

each [5'-X domain] said X domain comprises a common [oligonucleotide] sequence that is neither complementary to nor specific for said multiple target DNA sequences, whereby primer-primer dimer formation and the synthesis of spurious amplification products are prevented; and

each [3'-Y domain] said Y domain comprises a unique [oligonucleotide] sequence, wherein said unique sequence is [each oligonucleotide] complementary to and specific for one of said multiple target DNA sequences [of interest] suspected to be present in said DNA sample(s), whereby primer-primer dimer formation and synthesis of spurious amplification products are prevented; and

[c)] b) detecting [the] said amplification products.

15. (Amended) [A] The method according to claim 14, wherein said multiple target DNA sequences [of interest] are located within different regions of a gene present in said DNA sample(s).

16. (Amended) [A] The method according to claim 14, wherein said multiple target DNA sequences [of interest] are located within multiple genes present in said DNA sample(s).

17. (Twice Amended) A [plurality] multiplicity of amplified target DNA sequences [of interest amplified and detected] produced according to the method of [claim 13]

a) simultaneously contacting a DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer each having a 5'X domain and a 3'Y domain, wherein

(i) said X domain in said first oligonucleotide DNA primer comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said X domain in said second oligonucleotide DNA primer comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement;

c) subjecting said sample(s) to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same conditions and cycling parameters, whereby a multiplicity of amplified target DNA sequences are obtained.

18. (Twice Amended) A [plurality] multiplicity of amplified target DNA sequences [of interest amplified and detected] produced according to the method of [claim 14] contacting a DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs having a 5'X domain, and a 3'Y domain, under single multiplex polymerase chain reaction conditions wherein coamplification of multiple target DNA sequences occurs in one or more cycles of identical melting, annealing and extending temperatures and times, wherein

each said X domain comprises a common sequence that is neither complementary to nor specific for said multiple target DNA sequences, whereby primer-primer dimer formation and the synthesis of spurious amplification products are prevented; and

each said Y domain comprises a unique sequence, wherein said unique sequence is complementary to and specific for one of said multiple target DNA sequences suspected to be present in said DNA sample, whereby primer-primer dimer formation and synthesis of spurious amplification products are prevented; and, whereby a multiplicity of amplified target DNA sequences are obtained.

REMARKS

The Claimed Invention

The claimed invention relates to oligonucleotide primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex